Ref #	Hits	Search Query	DBs	Default Operator	Plurals	Time Stamp
L4	830	(alpha adj amylase$1) same bacillus same (mutant$1 or variant$1)	US-PGPUB; USPAT	OR	OFF	2005/01/03 12:56
L5	2228	(mutant$1 or variant$1) near10 (stability or thermostabilty or calcium adj depend$8)	US-PGPUB; USPAT	OR	OFF	2005/01/03 12:58
L6	109	4 and 5	US-PGPUB; USPAT	OR	OFF	2005/01/03 12:58

priority to 2/5/96

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PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20040253702 A1

TITLE: Variants of beta-glucosidases

PUBLICATION-DATE: December 16, 2004

INVENTOR-INFORMATION:

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Lamsa, Michael	Davis	CA	US	
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APPL-NO: 10/ 836063

DATE FILED: April 30, 2004

RELATED-US-APPL-DATA:

non-provisional-of-provisional 60467767 20030502 US

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US-CL-CURRENT: 435/209, 435/252.3 , 435/320.1 , 435/6 , 435/69.1 , 510/320 , 536/23.2

ABSTRACT:

The present invention relates to variants of a parent beta-glucosidase, comprising a substitution at one or more positions corresponding to positions 142, 183, 266, and 703 of amino acids 1 to 842 of SEQ ID NO: 2 or corresponding to positions 142, 183, 266, and 705 of amino acids 1 to 844 of SEQ ID NO: 70, wherein the variant has beta-glucosidase activity. The present invention also relates to nucleotide sequences encoding the variant beta-glucosidases and to nucleic acid constructs, vectors, and host cells comprising the nucleotide sequences.

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims the benefit of U.S. Provisional Applications No. 60/467767, filed May 2, 2003, and 60/528342, filed Dec. 9, 2003, which applications are incorporated herein by reference.

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Detail Description Paragraph - DETX (17):

[0067] The term "improved chemical stability" is defined herein as a variant enzyme displaying retention of enzymatic activity after a period of incubation in the presence of a chemical or chemicals, either naturally occurring or synthetic, that reduce the enzymatic activity of the parent enzyme. Improved chemical stability may also result in variants better able to catalyze a reaction in the presence of such chemicals.

Detail Description Paragraph - DETX (294):

[0329] Amylases: Suitable amylases (.alpha. and/or .beta.) include those of bacterial or fungal origin. Chemically modified or protein engineered mutants are included. Amylases include, for example, .alpha.-amylases obtained from Bacillus, e.g., a special strain of Bacillus licheniformis, described in more detail in GB 1,296,839.

PGPUB-DOCUMENT-NUMBER: 20040253696

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20040253696 A1

TITLE: Fermentation processes and compositions

PUBLICATION-DATE: December 16, 2004

INVENTOR-INFORMATION:

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APPL-NO: 10/ 459315

DATE FILED: June 10, 2003

US-CL-CURRENT: 435/161

ABSTRACT:

The present invention provides improved fermentation processes, including for use in an ethanol production process. The improved fermentation processes include applying at least one fatty acid oxidizing enzyme (such as a lipoxygenase) in a fermentation process. The improved fermentation process may also involve the addition of various additional enzymes and growth stimulators for the fermenting microorganisms, including vitamins and mineral.

---------- KWIC --------

Detail Description Paragraph - DETX (74):

[0087] More preferably, the alpha-amylase is a Bacillus alpha-amylases, such as, derived from a strain of B. licheniformis, B. amyloliquefaciens, and B. stearothermophilus. Other alpha-amylases include alpha-amylase derived from a strain of the Bacillus sp. NCIB 12289, NCIB 12512, NCIB 12513 or DSM 9375, all of which are described in detail in WO 95/26397, and the alpha-amylase described by Tsukamoto et al., Biochemical and Biophysical Research Communications, 151 (1988), pp. 25-31. Other alpha-amylase variants and hybrids are described in WO 96/23874, WO 97/41213, and WO 99/19467. Other alpha-amylase includes alpha-amylases derived from a strain of Aspergillus, such as, Aspergillus oryzae and Aspergillus niger alpha-amylases. In a preferred embodiment, the alpha-amylase is a acid alpha-amylase. In a more preferred embodiment the acid alpha-amylase is an acid fungal alpha-amylase or an acid bacterial alpha-amylase. More preferably, the acid alpha-amylase is an acid fungal alpha-amylase derived from the genus Aspergillus. A commercially available acid fungal amylase is SP288 (available from Novozymes A/S, Denmark).

Detail Description Paragraph - DETX (83):

[0096] Other Aspergillus glucoamylase variants include variants to enhance the thermal stability: G137A and G139A (Chen et al. (1996), Prot. Engng. 9, 499-505); D257E and D293E/Q (Chen et al. (1995), Prot Engng. 8, 575-582); N182 (Chen et al. (1994), Biochem. J. 301, 275-281); disulphide bonds, A246C (Fierobe et al. (1996), Biochemistry, 35, 8698-8704; and introduction of Pro residues in position A435 and S436 (Li et al. (1997), Protein Engng. 10, 1199-1204. Other glucoamylases include Talaromyces glucoamylases, in particular, derived from Talaromyces emersonii (WO 99/28448), Talaromyces

leycettanus (U.S. Pat. No. Re. 32,153), Talaromyces duponti, Talaromyces thermophilus (U.S. Pat. No. 4,587,215). Bacterial glucoamylases contemplated include glucoamylases from the genus Clostridium, in particular C. thermoamylolyticum (EP 135,138), and C. thermohydrosulfuricum (WO 86/01831).

PGPUB-DOCUMENT-NUMBER: 20040253676

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20040253676 A1

TITLE: Method for producing sweetners and alcohol

PUBLICATION-DATE: December 16, 2004

INVENTOR-INFORMATION:

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APPL-NO: 10/ 779418

DATE FILED: February 12, 2004

RELATED-US-APPL-DATA:

child 10779418 A1 20040212

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child 10025648 20011219 US

parent division-of 09902188 20010710 US PENDING

child 09902188 20010710 US

parent continuation-of 09354191 19990715 US GRANTED

parent-patent 6297038 US

child 09354191 19990715 US

parent continuation-of 08600656 19960213 US GRANTED

parent-patent 6093562 US

child 08600656 19960213 US

parent continuation-of PCT/DK96/00056 19960205 US UNKNOWN

FOREIGN-APPL-PRIORITY-DATA:

COUNTRY	APPL-NO	DOC-ID	APPL-DATE
DK	0126/95	1995DK-0126/95	February 3, 1995
DK	0336/95	1995DK-0336/95	March 29, 1995
DK	1097/95	1995DK-1097/95	September 29, 1995
DK	1121/95	1995DK-1121/95	October 6, 1995

US-CL-CURRENT: 435/69.1, 435/204 , 435/252.3 , 435/320.1 , 510/320 , 536/23.2

ABSTRACT:

The present invention relates to variants of a parent .alpha.-amylase, which parent .alpha.-amylase (i) has an amino acid sequence selected from the amino acid sequences shown in SEQ ID No. 1, SEQ ID No. 2, SEQ ID No. 3, and SEQ ID No. 7, respectively; or (ii) displays at least 80% homology with one or more of these amino acid sequences; and/or displays immunological cross-reactivity with an antibody raised against an .alpha.-amylase having one of these amino acid sequences; and/or is encoded by a DNA sequence which hybridizes with the same probe as a DNA sequence encoding an .alpha.-amylase having one of these amino acid sequences; in which variant:
(a) at least one amino acid residue of the parent .alpha.-amylase has been deleted; and/or
(b) at least one amino acid residue of the parent .alpha.-amylase has been replaced by a different amino acid residue; and/or
(c) at least one amino acid residue has been inserted relative to the parent .alpha.-amylase; the variant having .alpha.-amylase activity and exhibiting at least one of the following properties relative to the parent .alpha.-amylase: increased thermostability; increased stability towards oxidation; and reduced Ca.sup.2+ dependency; with the proviso that the amino acid sequence of the variant is not identical to any of the amino acid sequences shown in SEQ ID No. 1, SEQ ID No. 2, SEQ ID No. 3 and SEQ ID No. 7, respectively.

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application is a division of U.S. application Ser. No. 10/025,648, filed Dec. 19, 2001, which is a division of U.S. patent application Ser. No. 09/902,188, filed on Jul. 10, 2001, which is a continuation of U.S. patent application Ser. No. 09/354,191, now U.S. Pat. No. 6,297,038, filed on Jul. 15, 1999, which is a continuation of U.S. patent application Ser. No. 08/600,656, now U.S. Pat. No. 6,093,562, filed on Feb. 13, 1996, which is a continuation of application serial no. PCT/DK96/00056, filed on Feb. 5, 1996, which claims priority under 35 U.S.C. 119 of Danish application serial nos. 0126/95, filed on Feb. 3, 1995, 0336/95, filed on Mar. 29, 1995, 1097/95, filed on Sep. 29, 1995, and 1121/95, filed on Oct. 6, 1995, the contents of which are fully incorporated herein by reference.

---------- KWIC ---------

Abstract Paragraph - ABTX (4):
(c) at least one amino acid residue has been inserted relative to the parent .alpha.-amylase; the variant having .alpha.-amylase activity and exhibiting at least one of the following properties relative to the parent .alpha.-amylase: increased thermostability; increased stability towards oxidation; and reduced Ca.sup.2+ dependency; with the proviso that the amino acid sequence of the variant is not identical to any of the amino acid sequences shown in SEQ ID No. 1, SEQ ID No. 2, SEQ ID No. 3 and SEQ ID No. 7, respectively.

Summary of Invention Paragraph - BSTX (9):
[0008] WO 91/00353 discloses .alpha.-amylase mutants which differ from their parent .alpha.-amylase in at least one amino acid residue. The .alpha.-amylase mutants disclosed in said patent application are stated to exhibit improved properties for application in the degradation of starch and/or textile desizing due to their amino acid substitutions. Some of the mutants exhibit improved stability, but no improvements in enzymatic activity were reported or indicated. The only mutants exemplified are prepared from a parent B. licheniformis .alpha.-amylase and carry one of the following mutations: H133Y or H133Y+T149I. Another suggested mutation is A111T.

Summary of Invention Paragraph - BSTX (13):

[0012] EP 525 610 relates to mutant enzymes having improved stability towards ionic tensides (surfactants). The mutant enzymes have been produced by replacing an amino acid residue in the surface part of the parent enzyme with another amino acid residue. The only mutant enzyme specifically described in EP 525 610 is a protease. Amylase is mentioned as an example of an enzyme which may obtain an improved stability towards ionic tensides, but the type of amylase, its origin or specific mutations are not specified.

Summary of Invention Paragraph - BSTX (14):
[0013] WO 94/02597 discloses .alpha.-amylase mutants which exhibit improved stability and activity in the presence of oxidizing agents. In the mutant .alpha.-amylases, one or more methionine residues have been replaced with amino acid residues different from Cys and Met. The .alpha.-amylase mutants are stated to be useful as detergent and/or dishwashing additives as well as for textile desizing.

Summary of Invention Paragraph - BSTX (17):
[0016] An object of the present invention is to provide .alpha.-amylase variants which--relative to their parent .alpha.-amylase--possess improved properties of importance, inter alia, in relation to the washing and/or dishwashing performance of the variants in question, e.g. increased thermal stability, increased stability towards oxidation, reduced dependency on Ca.sup.2+ ion and/or improved stability or activity in the pH region of relevance in, e.g., laundry washing or dishwashing. Such variant .alpha.-amylases have the advantage, among others, that they may be employed in a lower dosage than their parent .alpha.-amylase. Furthermore, the .alpha.-amylase variants may be able to remove starchy stains which cannot, or can only with difficulty, be removed by .alpha.-amylase detergent enzymes known today.

Summary of Invention Paragraph - BSTX (92):
[0089] From the results obtained by the present inventors it appears that changes in a particular property, e.g. thermal stability or oxidation stability, exhibited by a variant relative to the parent .alpha.-amylase in question can to a considerable extent be correlated with the type of, and positioning of, mutation(s) (amino acid substitutions, deletions or insertions) in the variant. It is to be understood, however, that the observation that a particular mutation or pattern of mutations leads to changes in a given property in no way excludes the possibility that the mutation(s) in question can also influence other properties.

Summary of Invention Paragraph - BSTX (93):
[0090] Oxidation stability: With respect to increasing the oxidation stability of an .alpha.-amylase variant relative to its parent .alpha.-amylase, it appears to be particularly desirable that at least one, and preferably multiple, oxidizable amino acid residue(s) of the parent has/have been deleted or replaced (i.e. substituted by) a different amino acid residue which is less susceptible to oxidation than the original oxidizable amino acid residue.

Summary of Invention Paragraph - BSTX (94):
[0091] Particularly relevant oxidizable amino acid residues in this connection are cysteine, methionine, tryptophan and tyrosine. Thus, for example, in the case of parent .alpha.-amylases containing cysteine it is anticipated that deletion of cysteine residues, or substitution thereof by less oxidizable amino acid residues, will be of importance in obtaining variants with improved oxidation stability relative to the parent .alpha.-amylase.

Summary of Invention Paragraph - BSTX (95):
[0092] In the case of the above-mentioned parent .alpha.-amylases having the

amino acid sequences shown in SEQ ID No. 1, SEQ ID No. 2 and SEQ ID No. 7, respectively, all of which contain no cysteine residues but have a significant methionine content, the deletion or substitution of methionine residues is particularly relevant with respect to achieving improved oxidation stability of the resulting variants. Thus, deletion or substitution [e.g. by threonine (T), or by one of the other amino acids listed above] of one or more of the methionine residues in positions M9, M10, M105, M202, M208, M261, M309, M382, M430 and M440 of the amino acid sequences shown in SEQ ID No. 1, SEQ ID No. 2 and SEQ ID No. 7, and/or in position M323 of the amino acid sequence shown in SEQ ID No. 2 (or deletion or substitution of methionine residues in equivalent positions in the sequence of another .alpha.-amylase meeting one of the other criteria for a parent .alpha.-amylase mentioned above) appear to be particularly effective with respect to increasing the oxidation stability.

Summary of Invention Paragraph - BSTX (104):
[0101] Thermal stability: With respect to increasing the thermal stability of an .alpha.-amylase variant relative to its parent .alpha.-amylase, it appears to be particularly desirable to delete at least one, and preferably two or even three, of the following amino acid residues in the amino acid sequence shown in SEQ ID No. 1 (or their equivalents): F180, R181, G182, T183, G184 and K185. The corresponding, particularly relevant (and equivalent) amino acid residues in the amino acid sequences shown in SEQ ID No. 2, SEQ ID No. 3 and SEQ ID No. 7, respectively, are: F180, R181, G182, D183, G184 and K185 (SEQ ID No. 2); F178, R179, G180, I181, G182 and K183 (SEQ ID No. 3); and F180, R181, G182, H183, G184 and K185 (SEQ ID No. 7).

Summary of Invention Paragraph - BSTX (112):
[0109] Examples of specific mutations which appear to be of importance in connection with the thermal stability of an .alpha.-amylase variant relative to the parent .alpha.-amylase in question are one or more of the following substitutions in the amino acid sequence shown in SEQ ID No. 1 (or equivalents thereof in another parent .alpha.-amylase in the context of the invention): K269R; P260E; R124P; M105F,I,L,V; M208F,W,Y; L2171; V206I,L,F.

Summary of Invention Paragraph - BSTX (116):
[0113] Still further examples of mutations which appear to be of importance, inter alia, in achieving improved thermal stability of an .alpha.-amylase variant relative to the parent .alpha.-amylase in question are one or more of the following substitutions in the amino acid sequences shown in SEQ ID No. 1, SEQ ID No. 2 and SEQ ID No. 7 (or equivalents thereof in another parent .alpha.-amylase in the context of the invention): A354C+V479C; L351C+M430C; N457D,E+K385R; L355D,E+M430R,K; L355D,E+I411R,K; and N457D,E.

Summary of Invention Paragraph - BSTX (209):
[0206] .alpha.-Amylase activity is detected by Cibacron Red labelled amylopectin, which is immobilized on agarose. For screening for variants with increased thermal and high-pH stability, the filter with bound .alpha.-amylase variants is incubated in a buffer at pH 10.5 and 60.quadrature. or 65.quadrature.C for a specified time, rinsed briefly in deionized water and placed on the amylopectin-agarose matrix for activity detection. Residual activity is seen as lysis of Cibacron Red by amylopectin degradation. The conditions are chosen to be such that activity due to the .alpha.-amylase having the amino acid sequence shown in SEQ ID No. 1 can barely be detected. Stabilized variants show, under the same conditions, increased color intensity due to increased liberation of Cibacron Red.

Summary of Invention Paragraph - BSTX (219):
[0216] In the vector, the DNA sequence should be operably connected to a suitable promoter sequence. The promoter may be any DNA sequence which shows

transcriptional activity in the host cell of choice and may be derived from genes encoding proteins either homologous or heterologous to the host cell. Examples of suitable promoters for directing the transcription of the DNA sequence encoding an .alpha.-amylase variant of the invention, especially in a bacterial host, are the promoter of the lac operon of E. coli, the Streptomyces coelicolor agarase gene dagA promoters, the promoters of the Bacillus licheniformis .alpha.-amylase gene (amyL), the promoters of the Bacillus stearothermophilus maltogenic amylase gene (amyM), the promoters of the Bacillus Amyloliquefaciens .alpha.-amylase (amyQ), the promoters of the Bacillus subtilis xylA and xylB genes etc. For transcription in a fungal host, examples of useful promoters are those derived from the gene encoding A. oryzae TAKA amylase, Rhizomucor miehei aspartic proteinase, A. niger neutral .alpha.-amylase, A. niger acid stable .alpha.-amylase, A. niger glucoamylase, Rhizomucor miehei lipase, A. oryzae alkaline protease, A. oryzae triose phosphate isomerase or A. nidulans acetamidase.

Detail Description Paragraph - DETX (22):
[0330] It is apparent that the each of the tested variants T183*+G184* (which exhibits, inter alia, higher thermal stability than the parent .alpha.-amylase), Y243F (which exhibits lower calcium ion dependency than the parent .alpha.-amylase) and K269R (which exhibits lower calcium ion dependency and higher stability at high pH than the parent .alpha.-amylase) exhibits significantly improved dishwashing performance relative to the parent .alpha.-amylase.

Detail Description Paragraph - DETX (59):
[0365] Determination of Oxidation Stability of M202 Substitution Variants of the Parent .alpha.-Amylases Having the Amino Acid Sequences Shown in SEQ ID No. 1 and SEQ ID No. 2

Detail Description Paragraph - DETX (60):
[0366] A: Oxidation Stability of Variants of the Sequence in SEQ ID No. 1

Detail Description Paragraph - DETX (64):
[0370] All the M202 substitution variants tested clearly exhibit significantly improved stability towards oxidation relative to the parent .alpha.-amylase (SEQ ID No. 1).

Detail Description Paragraph - DETX (65):
[0371] B: Oxidation Stability of Variants of the Sequence in SEQ ID No. 2

Detail Description Paragraph - DETX (67):
[0373] The two "substitution+pairwise deletion" variants tested (which both comprise an M202 substitution) clearly exhibit significantly improved stability towards oxidation relative to the parent .alpha.-amylase (SEQ ID No. 2).

Detail Description Paragraph - DETX (69):
[0374] Determination of Thermal Stability of Variants of the Parent .alpha.-Amylases Having the Amino Acid Sequences Shown in SEQ ID No. 1 and SEQ ID No. 2

Detail Description Paragraph - DETX (70):
[0375] A: Thermal Stability of Pairwise Deletion Variants of the Sequence in SEQ ID No. 1

Detail Description Paragraph - DETX (78):
[0383] It is apparent that all of the pairwise deletion variants tested exhibit significantly improved thermal stability relative to the parent .alpha.-amylase (SEQ ID No. 1), and that the thermal stability of Variant 5,

which in addition to the pairwise deletion mutation of Variant 4 comprises the substitution R124P, is markedly higher than that of the other variants. Since calorimetric results for the substitution variant R124P (comprising only the substitution R124P) reveal an approximately 7.degree. C. thermostabilization thereof relative to the parent .alpha.-amylase, it appears that the thermostabilizing effects of the mutation R124P and the pairwise deletion, respectively, reinforce each other.

Detail Description Paragraph - DETX (79):
[0384] B: Thermal **Stability of Pairwise Deletion Variants** of the Sequence in SEQ ID No. 2

Detail Description Paragraph - DETX (84):
[0389] Again, it is apparent that the pairwise deletion **variants in question exhibit significantly improved thermal stability** relative to the parent .alpha.-amylase (SEQ ID No. 2).

Detail Description Paragraph - DETX (85):
[0390] C: Thermal **Stability of a Multi-Combination Variant** of the Sequence in SEQ ID No. 1

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PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20040229764 A1

TITLE: Fungamyl-like alpha-amylase variants

PUBLICATION-DATE: November 18, 2004

INVENTOR-INFORMATION:

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Pedersen, Sven	Gentofte		DK	

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DATE FILED: April 7, 2004

RELATED-US-APPL-DATA:

child 10820200 A1 20040407

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non-provisional-of-provisional 60165786 19991116 US

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COUNTRY	APPL-NO	DOC-ID	APPL-DATE
DK	PA 1999 01617	1999DK-PA 1999 01617	November 10, 1999

US-CL-CURRENT: 510/320, 435/203 , 435/252.3 , 435/6 , 435/69.1 , 536/23.2

ABSTRACT:

The invention relates to a variant of a parent Fungamyl-like fungal alpha-amylase, which exhibits improved thermal stability at acidic pH suitable for, e.g., starch processes.

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application is a Continuation of U.S. application Ser. No. 09/710,339, filed Nov. 09, 2000, (now allowed), which claims, under 35 U.S.C. 119, the benefit of U.S. provisional application No. 60/165,786, filed Nov. 16, 1999, and priority from Danish application no. PA 1999 01617 filed Nov. 10, 1999, the contents of which are fully incorporated herein by reference.

---------- KWIC ---------

Abstract Paragraph - ABTX (1):
The invention relates to a variant of a parent Fungamyl-like fungal alpha-amylase, which exhibits improved thermal stability at acidic pH suitable for, e.g., starch processes.

Summary of Invention Paragraph - BSTX (2):
[0002] The present invention relates to alpha-amylase variants (mutants) of

Fungamyl.TM.-like alpha-amylases, in particular with improved thermal stability at acidic pH. The invention also relates to the use of such variants.

Summary of Invention Paragraph - BSTX (130):
[0127] That a Fungamyl-like alpha-amylase variant is more acidic than the parent Fungamyl-like alpha-amylase means that the stability at acidic pH is higher that for the corresponding parent alpha-amylase. That the amylase is more acidic may be determined as described in the "Materials & Methods" section.

Summary of Invention Paragraph - BSTX (141):
[0138] In a preferred embodiment the variant of the invention has improved thermal stability, in particular at acidic pH.

Summary of Invention Paragraph - BSTX (156):
[0153] In relation to the above, a further aspect of the present invention relates to a method for generating a variant of a parent Fungamyl-like alpha-amylase, wherein the variant exhibits increased thermal stability, especially at acidic pH, relative to the parent, the method comprising:

Summary of Invention Paragraph - BSTX (159):
[0156] (c) screening for host cells expressing an alpha-amylase variant which has an altered property (i.e., thermal stability) relative to the parent Fungamyl-like alpha-amylase.

Summary of Invention Paragraph - BSTX (181):
[0178] Examples of suitable promoters for directing the transcription of the DNA sequence encoding an alpha-amylase variant of the invention, especially in a bacterial host, are the promoter of the lac operon of E. coli, the Streptomyces coelicolor agarase gene dagA promoters, the promoters of the Bacillus licheniformis alpha-amylase gene (amyL), the promoters of the Bacillus stearothermophilus maltogenic amylase gene (amyM), the promoters of the Bacillus amiyloliquefaciens alpha-amylase (amyQ), the promoters of the Bacillus subtilis xylA and xylB genes etc. For transcription in a fungal host, examples of useful promoters are those derived from the gene encoding A. oryzae TAKA amylase, the TPI (triose phosphate isomerase) promoter from S. cerevisiae (Alber et al. (1982), J. Mol. Appl. Genet 1, p. 419-434, Rhizo-mucor miehei aspartic proteinase, A. niger neutral alpha-amylase, A. niger acid stable alpha-amylase, A. niger glucoamylase, Rhizo-mucor miehei lipase, A. oryzae alkaline protease, A. oryzae triose phosphate isomerase or A. nidulans acetamidase.

Summary of Invention Paragraph - BSTX (204):
[0201] By improving the thermo stability of the Fungamyl-like alpha-amylase variant according to the invention said alpha-amylases may be used for starch liquefaction.

Summary of Invention Paragraph - BSTX (265):
[0262] Thermal/pH Stability Determination of Variant of the Invention

Summary of Invention Paragraph - BSTX (266):
[0263] The thermal stability of variants of the invention is tested using the following method: 950 micro liter 0.1 M Citrate+4.3 mM Ca.sup.2+ buffer is incubated for 1 hour at 60.degree. C. 50 micro liter enzyme in buffer (4 AFAU/ml) is added. 2.times.40 micro liter samples are taken at 0 and 60 minutes and chilled on ice. The activity (AFAU/ml) measured before incubation (0 minutes) is used as reference (100%). The decline in percent is calculated as a function of the incubation time.

Summary of Invention Paragraph - BSTX (273):
[0270] 3. Decide on which kind of mutations should be carried out, e.g. with respect to the desired stability and/or performance of the variant to be constructed,

Detail Description Paragraph - DETX (17):
[0294] The variant constructed in Example 1 is tested for increased thermostability in accordance with the thermo stability determination assay disclosed in the "Materials & Methods" section.

Detail Description Paragraph - DETX (20):
[0296] The variant constructed in Example 1 is tested for increased stability at acidic pH in accordance with the pH stability determination assay disclosed in the "Materials & Methods" section.

Claims Text - CLTX (4):
33. The variant of claim 31, wherein the variant has improved thermostability and/or increased stability at acidic pH.

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PGPUB-FILING-TYPE: new

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TITLE: Novel subtilases

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INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
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DK	PA 2003 00689	2003DK-PA 2003 00689	May 7, 2003
DK	PA 2003 00119	2003DK-PA 2003 00119	January 30, 2003

US-CL-CURRENT: 435/222

ABSTRACT:

The present invention relates to methods for producing variants of a parent TY145 subtilase and of a parent BPN' subtilase and to TY145 and BPN' variants having altered properties as compared to the parent TY145/BPN' subtilase.

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application is a continuation of PCT/DK2004/000066 filed Jan. 30, 2004, which claims priority or the benefit under 35 U.S.C. 119 of Danish application nos. PA 2003 00119 and PA 2003 00689 filed Jan. 30, 2003 and May 7, 2003, respectively, and U.S. Provisional Application Nos. 60/445,300 and 60/468,574 filed Feb. 5, 2003 and May 7, 2003, respectively, the contents of which are fully incorporated herein by reference.

---------- KWIC ---------

Summary of Invention Paragraph - BSTX (5):

[0004] The present invention relates to variants of TY145 subtilases and BPN' subtilases and to methods of construction such variants with altered properties, such as stability (e.g. thermostability or storage stability),

Ca.sup.2+ dependency, pH dependent activity.

Detail Description Paragraph - DETX (131):
[0155] In detergent compositions calcium chelaters contribute to removal of calcium from the subtilases with subsequent inactivation of the enzyme as the result. To decrease the inactivation due to calcium removal of e.g. calcium chelaters, variants with improved calcium stability can be constructed.

Detail Description Paragraph - DETX (142):
[0166] A variant with improved stability (typically increased thermostability) may be obtained by substitution with proline, introduction of a disulfide bond, altering a hydrogen bond contact, altering charge distribution, introduction of a salt bridge, filling in an internal structural cavity with one or more amino acids with bulkier side groups (in e.g. regions which are structurally mobile), substitution of histidine residues with other amino acids, removal of a deamidation site, or by helix capping.

Detail Description Paragraph - DETX (166):
[0190] A TY145 variant of the present invention with improved stability, e.g. thermostability, as compared to the parent TY145 may be obtained by introducing new inter-domain or intra-domain bonds, such as by establishing inter- or intra-domain disulfide bridges.

Detail Description Paragraph - DETX (171):
[0195] A variant with improved stability (typically improved thermostability) as compared to the parent subtilase may be obtained by changing the surface charge distribution of the subtilase. For example, when the pH is lowered to about 5 or below histidine residues typically become positively charged and, consequently, unfavorable electrostatic interactions on the protein surface may occur. By engineering the surface charge of the subtilase one may avoid such unfavorable electrostatic interactions that in turn lead to a higher stability of the subtilase.

Detail Description Paragraph - DETX (178):
[0202] f) testing the stability of said variant; and

Detail Description Paragraph - DETX (180):
[0204] h) selecting a subtilase variant having increased stability as compared to the parent subtilase.

Detail Description Paragraph - DETX (261):
[0285] In relation to the above, a further aspect of the present invention relates to a method for generating a variant of a parent subtilase, wherein the variant exhibits an altered property, such as increased thermostability, increased stability at low pH and at low calcium concentration, relative to the parent subtilase, the method comprising:

Detail Description Paragraph - DETX (283):
[0307] 3. Decide on which kind of mutations should be carried out, e.g. with respect to the desired stability and/or performance of the variant to be constructed

Detail Description Paragraph - DETX (340):
[0364] Amylases: Suitable amylases (.alpha. and/or .beta.) include those of bacterial or fungal origin. Chemically modified or protein engineered mutants are included. Amylases include, for example, .alpha.-amylases obtained from Bacillus, e.g. a special strain of B. licheniformis, described in more detail in GB 1,296,839.

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ABSTRACT:

The present invention relates to an improved process for producing a fermentation product.

---------- KWIC ---------

Detail Description Paragraph - DETX (85):
[0090] Other contemplated Aspergillus glucoamylase variants include variants to enhance the thermal stability: G137A and G139A (Chen et al. (1996), Prot Engng. 9, 499-505); D257E and D293E/Q (Chen et al. (1995), Prot. Engng. 8, 575-582); N182 (Chen et al. (1994), Biochem. J. 301, 275-281); disulphide bonds, A246C (Fierobe et al. (1996), Biochemistry, 35, 8698-8704; and introduction of Pro residues in position A435 and S436 (Li et al. (1997), Protein Engng. 10, 1199-1204. Furthermore Clark Ford presented a paper on Oct. 17, 1997, ENZYME ENGINEERING 14, Beijing/China Oct. 12-17, 1997, Abstract number: Abstract book p. 0-61. The abstract suggests mutations in positions G137A, N20C/A27C, and S30P in an Aspergillus awamori glucoamylase to improve the thermal stability. Other glucoamylases include Talaromyces glucoamylases, in particular derived from Talaromyces emersonii (WO 99/28448), Talaromyces leycettanus, Talaromyces duponti (U.S. Pat. No. 32,153), Talaromyces thermophilus (U.S. Pat. No. 4,587,215). Bacterial glucoamylases contemplated include glucoamylases from the genus Clostridium, in particular C. thermoamylolyticum (EP 135,138), and C. thermohydrosulfuricum (WO 86/01831).

Detail Description Paragraph - DETX (107):

[0112] The liquefaction step may be performed in the presence of an alpha-amylase derived from a microorganism or a plant. Preferred alpha-amylases are of fungal or bacterial origin. Bacillus alpha-amylases (often referred to as "Termamyl-like alpha-amylases"), variant and hybrids thereof, are specifically contemplated according to the invention. Well-known Termamyl-like alpha-amylases include alpha-amylase derived from a strain of B. licheniformis (commercially available as Termamyl.TM.), B. amyloliquefaciens, and B. stearothermophilus alpha-amylase (BSG). Other Termamyl-like alpha-amylases include alpha-amylase derived from a strain of the Bacillus sp. NCIB 12289, NCIB 12512, NCIB 12513 or DSM 9375, all of which are described in detail in WO 95/26397, and the alpha-amylase described by Tsukamoto et al., Biochemical and Biophysical Research Communications, 151 (1988), pp. 25-31. In the context of the present invention a Termamyl-like alpha-amylase is an alpha-amylase as defined in WO 99/19467 on page 3, line 18 to page 6, line 27. Contemplated variants and hybrids are described in WO 96/23874, WO 97/41213, and WO 99/19467. Contemplated alpha-amylase derived from a strain of Aspergillus includes Aspergillus oryzae and Aspergillus niger--amylases.

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ABSTRACT:

The present invention relates to variants (mutants) of parent Termamyl-like alpha-amylases, which variant has alpha-amylase activity and exhibits altered properties relative to the parent alpha-amylase.

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Detail Description Paragraph - DETX (622):
[0670] Variants of the invention may have altered oxidation stability, in particular higher oxidation stability, in comparison to the parent alpha-amylase. Increased oxidation stability is advantageous in, e.g., detergent compositions and descresed oxidation stability may be advantageous in composition for starch liquefaction. Oxidation stability may be determined as described in the "Material & Methods" section below.

Detail Description Paragraph - DETX (1373):
[1421] In an aspect, the invention relates to providing alpha-amylase variants with reduced sensitivity (or improved stability against denaturation) to anionic surfactants (in particular linear alkyl sulphonates (LAS)). These variants are provided by substituting, deleting or inserting an amino acid residue in the parent alpha-amylase as indicated below with a more hydrophilic amino acid residue. Such variants may be prepared by:

Detail Description Paragraph - DETX (1378):
[1426] Variants of the invention with reduced sensitivity to anionic surfactants, in particular linear alkyl sulphonates (LAS), include (using the Bacillus licheniformis alpha-amylase shown in SEQ ID NO: 8 numbering):

Detail Description Paragraph - DETX (1722):
[1770] In an aspect the invention relates to Termamyl-like alpha-amylase variant with increased stability at acidic pH and/or at high temperature in comparison to the parent alpha-amylase. Such variants are especially suitable for starch liquefaction.

Detail Description Paragraph - DETX (2155):
[2203] In the vector, the DNA sequence should be operably connected to a suitable promoter sequence. The promoter may be any DNA sequence, which shows transcriptional activity in the host cell of choice and may be derived from genes encoding proteins either homologous or heterologous to the host cell. Examples of suitable promoters for directing the transcription of the DNA sequence encoding an alpha-amylase variant of the invention, especially in a bacterial host, are the promoter of the lac operon of E.coli, the Streptomyces coelicolor agarase gene dagA promoters, the promoters of the Bacillus licheniformis alpha-amylase gene (amyL), the promoters of the Bacillus stearothermophilus maltogenic amylase gene (amyM), the promoters of the Bacillus amyloliquefaciens alpha-amylase (amyQ), the promoters of the Bacillus subtilis xylA and xylB genes etc. For transcription in a fungal host, examples of useful promoters are those derived from the gene encoding A. oryzae TAKA amylase, Rhizomucor miehei aspartic proteinase, A. niger neutral alpha-amylase, A. niger acid stable alpha-amylase, A. niger glucoamylase, Rhizomucor miehei lipase, A. oryzae alkaline protease, A. oryzae triose phosphate isomerase or A. nidulans acetamidase.

Detail Description Paragraph - DETX (2233):
[2281] Amylases: Suitable amylases (alpha and/or beta) include those of bacterial or fungal origin. Chemically modified or protein engineered mutants are included. Amylases include, for example, alpha-amylases obtained from Bacillus, e.g., a special strain of B. licheniformis, described in more detail in GB 1,296,839. Examples of useful alpha-amylases are the variants described in WO 94/02597, WO 94/18314, WO 96/23873, and WO 97/43424, especially the variants with substitutions in one or more of the following positions: 15, 23, 105, 106, 124, 128, 133, 154, 156, 181, 188, 190, 197, 202, 208, 209, 243, 264, 304, 305, 391, 408, and 444.

Detail Description Paragraph - DETX (2291):
[2339] The below assays can be used to screening of Termamyl-like alpha-amylase variants having altered stability at high or low pH and/or under Ca.sup.2+ depleted conditions compared to the parent enzyme and Termamyl-like alpha-amylase.

Detail Description Paragraph - DETX (2303):
[2351] Stability Assay of Unpurified Variants

Detail Description Paragraph - DETX (2304):

[2352] Bacillus cultures expressing the variants to be analysed are grown for 21 hours at 37.degree. C. in 10 ml LB+chloramphenicol. 800 micro liter culture is mixed with 200 micro l citrate buffer, pH 4.5. A number of 70 micro l aliquots corresponding to the number of sample time points are made in PCR tubes and incubated at 70.degree. C. or 90.degree. C. for various time points (typically 5, 10, 15, 20, 25 and 30 minutes) in a PCR machine. The 0 min sample is not incubated at high temperature. Activity in the sample is measured by transferring 20 micro l to 200 micro l of the alpha-amylase PNP-G.sub.7 substrate MPR3 ((Boehringer Mannheim Cat. no. 1660730) as described below under "Assays for Alpha-Amylase Activity". Results are plotted as percentage activity (relative to the 0 time point) versus time, or stated as percentage residual activity after incubation for a certain period of time.

Detail Description Paragraph - DETX (2310):
[2358] Stability Determination of Purified Variants

Detail Description Paragraph - DETX (2311):
[2359] All stability trials of purified variants are made using the same set up. The method is as follows:

Detail Description Paragraph - DETX (2360):
[2406] The below listed variants are constructed as described in EXAMPLE 1 of WO 00/29560 (from Novozymes A/S) in the parent Bacillus licheniformis alpha-amylase shown in SEQ ID NO: 8.

Detail Description Paragraph - DETX (2790):
[2835] The below listed variants are constructed as described in EXAMPLE 1 of WO 00/37626 (from Novozymes A/S) in the parent Bacillus licheniformis alpha-amylase shown in SEQ ID NO: 8. The alterations of the variants are, as specified in the list below, insertion of an amino acid downstream of the amino acid which occupies the position, or deletion of the amino acid which occupies the position.

Claims Text - CLTX (1):
1. A variant of a parent Termamyl-like alpha-amylase, comprising an alteration at one or more positions selected from the group of: 5, 6, 36, 37, 38, 39, 42, 45, 47, 63, 66, 69, 70, 71, 72, 74, 75, 76, 79, 82, 83, 86, 87, 89, 93, 112, 113, 117, 120, 137, 213, 216, 220, 223, 225, 226, 227, 229, 243, 245, 279, 282, 311, 321, 324, 352, 353, 354, 357, 361, 362, 364, 368, 390, 395, 397, 399, 400, 401, 425, 451, 452, 453, 466, 468, 470, 471, 478, wherein (a) the alteration(s) are independently (i) an insertion of an amino acid downstream of the amino acid which occupies the position, (ii) a deletion of the amino acid which occupies the position, or (iii) a substitution of the amino acid which occupies the position with a different amino acid, (b) the variant has alpha-amylase activity and (c) each position corresponds to a position of the amino acid sequence of the parent termamyl-like alpha-amylase having the amino acid sequence shown in SEQ ID NO: 8 (bacillus licheniformis alph-amylase).

Claims Text - CLTX (2):
2. A variant of a parent Termamyl-like alpha-amylase, comprising one or more of the following substitutions. X1A,R,N,D,C,Q,E,G,H,I,L,K,M,F,P,S,T,-W,Y; X2R,N,D,C,Q,E,G,H,I,L,K,M,F,S,T,W,Y,V; X3A,R,N,D,C,Q,E,G,H,I,L,K,M,F,-P,S,T,W,Y; X4A,R,N,D,C,Q,E,G,H,I,L,K,M,F,P,S,T,W,Y,V; X13R,N,D,C,Q,E,G,H,K,M,P,S,T,W; X14A,R,D,C,G,K,M,P,W; X16R,N,D,C,Q,E,G,H,I,L,K,M,F,S,T,W,Y,V; X17A,R,N,D,C,Q,E,G,H,I,L,K,M,F,P,-S,T,W,Y,V; X18A,R,N,D,C,Q,E,G,H,I,L,K,M,F,P,S,T,W,Y,V; X20A,R,N,D,C,Q,E,G,H,I,L,K,M,F,P,S,T,W,Y,V; X23A,R,N,D,C,Q,E,G,H,I,L,M,F,-P,S,W,Y,V; X24A,R,N,D,C,Q,E,G,H,I,L,K,M,F,P,S,T,W,Y,V; X26A,D,C,E,G,H,I,L,M,F,P,S,T,W,V; X34A,R,N,C,Q,E,G,H,I,L,K,M,F,P,T,W,Y,V;

X35A,R,N,D,C,Q,E,G,H,K,M,F,P,S,T,W,Y,V; X49A,C,G,H,P,T;
X50A,R,N,C,Q,E,G,H,K,M,F,P,S,W; X51A,N,D,C,Q,E,G,H,I,L,M,F,P,S,T,W,Y,V;
X52A,R,D,C,Q,E,G,H,K,P; X53A,D,C,G,H,K,M,P; X61A,R,N,D,C,Q,E,G,H,I,L,K,M,-
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X125R,N,Q,E,G,I,K,M,F,S,T,W,Y; X126N,Q,G,H,I,L,M,F,P,S,T,W,Y,V;
X127A,R,N,D,E,G,H,I,L,K,M,F,P,S,T,W,Y,V; X128A,R,N,D,C,Q,G,H,I,L,K,M,F,P,-
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X134A,N,D,C,E,G,H,I,L,K,M,F,P,S,T,W,Y,V; X136A,R,N,D,C,E,G,H,I,L,K,M,F,P,-
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X164R,I,L,M,F,P,S,T,W,Y,V; X165A,N,D,C,Q,E,H,I,L,K,M,F,P,S- ,T,W,Y,V;
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X191A,R,N,Q,G,H,I,L,M,F,P,S,T,W,Y,V; X193A,R,G,M,P,W,Y;
X196A,N,Q,G,H,I,L,M,P,S,T,W,V; X198A,R,G,M,P,W; X204R,L,M,F,P,T,W,Y,V;
X205A,G,H,I,L,M,F,P,W,Y,V; X206R,N,D,C,Q,E,G,H,I,L,K,M,F,P,S,T,W,Y,V;
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X214A,D,C,Q,E,G,I,L,M,F,P,S,T,Y,V; X217A,R,N,D,C,Q,G,H,I,L,M,F,P,S,T,W,Y;
X218A,R,N,D,C,Q,E,G,H,I,L,K,M,F,P,S,T,W,Y,V; X221A,R,D,C,Q,E,G,H,I,L,K,M,-
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X266A,R,N,D,C,Q,E,G,H,I,L,K,M,F,P,S,T,W,Y,V; X267A,R,N,D,C,Q,E,G,H,K,P,S,-
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X269A,N,C,Q,G,H,I,L,M,F,P,S,T,W,Y,V; X271A,R,N,D,C,Q,E,G,H,I,L,K,M,F,P,S,-
T,W,Y,V; X272A,R,N,D,C,Q,E,G,H,I,L,K,M,F,P,S,T,W,Y,V;
X275A,R,D,C,Q,E,G,H,I,L,K,M,F,P,S,W,Y,V; X276A,R,N,D,C,Q,E,G,H,I,L,M,F,P,-
S,T,W,Y,V; X278A,R,N,D,C,Q,E,G,H,I,L,K,M,F,P,S,T,W,Y,V;

X280A,R,D,C,E,G,H,I,L,K,M,F,P,W,Y,V; X290W,Y; X291A,R,N,Q,G,H,I,L,M,F,P,S-
,T,W,Y,V; X293A,R,N,G,I,L,M,P,S,T,W,V; X294R,N,Q,G,H,I,L,M,F,P,S,T,W,Y;
X297A,G,H,I,L, M, F,P,W,Y,V; X298G,H,I,L,M,F,P,S,T,W,Y,V; X299A,G,H,M,P,S,T;
X300A,C,G,H,I,L,M,F,P,T,W,Y,V; X301 N,Q,H,I,L,M,F,P,S,T,W,Y,V; X302R,M,P,W,Y;
X303R,I,L,M,F,P,S,T,W,Y,V; X305G,I,L,M,F,P,S,T,W,Y,V;
X306Q,G,H,I,L,M,F,P,S,T,W,Y,V; X308A,R,N,D,C,Q,E,G,H,I,L,K,M,F,P,S,T,W,Y,V;
X309N,Q,G,H,I,L,M,F,P,S,T,W,- Y,V; X310A,R,N,C,Q,E,H,I,L,K,M,F,P,S,T,W,Y,V;
X314A,D,C,E,G,I,L,M,F,P,W,Y,- V; X315A,N,C,Q,E,G,H,I,L,M,F,P,S,T,W,Y,V;
X316A,R,N,D,C,Q,E,G,H,I,L,K,M,F,- P,S,T,W,V;
X317R,N,D,C,Q,E,G,H,I,L,K,M,F,P,S,T,W,Y,V;
X318A,R,N,D,C,Q,E,G,H,I,K,P,S,W,Y,V; X319A,R,N,D,C,Q,E,G,H,I,L,M,F,P,S,T,-
W,Y,V; X328A,R,N,D,C,Q,E,G,H,I,L,K,M,F,P,S,T,W,Y,V;
X332A,R,N,C,Q,E,G,H,I,L,K,M,F,P,S,T,W,Y,V; X333A,N,D,C,G,I,M,F,P,S,T,Y,V;
X334R,N,C,Q,E,G,H,K,M,F,P,S,W,Y; X335R,D,C,Q,E,H,I,L,K,M,F,P,W,Y,V;
X336A,N,D,C,Q,E,G,H,I,L,M,F,P,S,T,W,Y,V; X337A,R,N,C,Q,E,G,H,I,L,M,F,P,S,-
T,W,Y,V; X338A,R,N,C,Q,G,H,I,L,K,M,F,P,S,T,W,Y,V; X340I,L,M,F,P,S,T,W,Y,V;
X341A,R,D,C,G,H,I,L,K,M,F,W,Y,V; X342R,I,L,M,F,P,S,T,W,Y,V;
X345R,N,D,C,Q,E,G,H,I,L,K,M,F,P,T,W,Y,V; X355A,R,N,D,C,Q,G,H,I,L,K,M,F,P,-
S,T,W,Y,V; X358A,R,D,C,G,K,M,P,W,Y; X363A,R,D,C,G,K,M,P,W,Y;
X370A,R,N,D,C,Q,E,G,H,I,L,K,M,F,P,S,T,W,Y,V; X371A,N,C,Q,E,G,H,I,L,K,M,F,-
P,S,T,W,Y,V; X373A,R,N,D,C,Q,E,G,H,I,L,K,M,F,S,T,W,Y,V;
X374A,N,D,C,Q,E,G,H,I,L,K,M,F,S,T,W,Y,V; X375A,R,N,D,C,Q,G,H,I,L,K,M,F,P,-
S,T,W,V; X376A,RN,D,C,Q,E,G,H,I,L,M,F,P,S,T,W,Y,V; X378R,N,D,C,Q,E,G,H,I,L-
,K,M,F,S,T,W,Y,V; X379A,R,N,D,C,Q,E,G,H,I,L,K,M,F,P,T,W,Y,V;
X381A,R,N,D,C,Q,E,G,H,I,L,K,M,F,P,S,T,W,Y,V; X389A,D,C,G,H,M,F,P,S,T,W,Y,- V;
X393A,N,C,Q,E,G,H,I,L,K,M,F,P,S,T,W,Y,V; X394A,R,D,C,G,K,M,P,W,Y;
X396A,R,D,C,G,K,M,P,W,Y; X398A,R,N,D,C,Q,E,G,H,I,L,M,F,W,Y,V;
X402R,C,G,K,M,P,W,Y; X403A,R,N,Q,G,H,I,L,M,F,P,S,T,W,Y,V;
X404R,I,L,M,F,P,S,T,W,Y,V; X405R,G,H,I,L,M,F,P,W,Y,V;
X406A,R,G,H,I,M,F,P,Y,V; X407A,R,Q,G,H,I,L,M,F,P,S,T,W,Y,V;
X408A,R,N,D,C,Q,E,G,H,I,K,M,P,S,T,W,Y,V; X413A,R,N,D,C,Q,E,G,H,I,L,K,M,F,-
P,S,T,W,Y,V; X414A,R,N,D,C,Q,E,G,H,I,L,K,M,F,P,S,T,W,Y,V;
X415A,R,N,D,C,Q,E,G,H,I,L,K,M,F,P,S,T,W,Y,V; X416A,R,N,D,C,Q,E,G,H,I,L,K,-
M,F,P,S,T,W,Y,V; X417A,R,N,D,C,Q,E,G,H,I,L,K,M,F,P,S,W,Y,V;
X418A,R,N,D,C,Q,E,G,H,I,L,K,M,F,P,S,T,W,Y,V; X419A,R,N,D,C,Q,E,G,H,I,L,M,-
F,P,S,T,W,Y,V; X420A,N,D,C,E,G,H,I,L,K,M,F,S,T,W,Y,V;
X421A,R,N,D,C,Q,E,H,I,L,K,M,F,P,S,T,W,Y,V; X422A,R,N,D,C,Q,E,G,H,I,L,K,M,-
F,P,S,T,W,Y,V; X431A,R,N,Q,G,H,I,L,M,F,P,S,T,W,Y,V;
X432G,H,I,L,M,F,P,S,T,W,Y,V; X433A,R,N,Q,G,H,I,L,M,F,P,S,T,W,Y,V;
X434A,R,N,Q,G,H,I,L,M,F,P,S,T,W,Y,V; X435Q,G,H,I,L,M,F,P,T,W,Y,V;
X436A,R,N,D,C,Q,E,G,H,I,L,K,M,F,P,S,T,W,Y,V; X437A,N,D,C,Q,E,G,H,i,L,K,M,-
F,P,S,T,W,Y,V; X439A,R,D,C,G,K,M,P,W,Y; X442A,R,N,D,C,E,G,H,I,L,M,F,P,S,T,-
W,Y,V; X443A,R,N,D,C,E,G,H,I,L,M,F,P,S,T,W,Y,V; X444A,C,G,H,I,L,M,F,P,S,T,-
W,Y,V; X445A,R,N,D,C,Q,E,G,H,I,L,K,M,F,P,S,T,W,Y,V;
X446A,R,N,D,C,Q,E,G,H,I,L,K,M,F,P,S,T,W,Y,V; X447A,N,D,C,G,H,I,L,M,F,P,S,-
T,W,Y,V; X448A,R,N,D,C,Q,E,G,H,I,L,K,M,F,P,S,T,W,Y,V;
X450A,N,D,C,Q,E,G,I,L,K,M,F,P,S,T,W,V; X454A,R,N,D,C,Q,E,G,H,I,L,K,M,F,P,-
S,T,W,Y,V; X455A,R,N,C,Q,G,H,I,L,K,M,F,P,S,T,W,Y,V;
X456A,D,C,E,G,H,I,L,M,F,P,S,T,W,Y,V; X457A,R,N,D,C,Q,E,G,H,I,L,K,M,F,W,Y,- V;
X458A,R,N,C,Q,E,G,H,I,L,K,M,F,P,S,T,W,Y,V; X459R,N,D,C,Q,E,G,H,I,L,K,M,-
F,S,W,Y,V; X460A,R,N,D,C,Q,E,G,H,I,L,K,M,F,P,S,T,W,Y,V;
X461A,R,N,D,C,Q,E,G,H,I,L,M,F,P,S,W,Y,V; X463A,R,N,D,C,Q,E,H,I,L,K,M,F,P,-
S,T,W,Y,V; X464A,R,N,D,C,Q,E,G,H,I,L,K,M,F,P,S,T,W,Y,V;
X465A,R,N,C,Q,E,G,H,I,L,K,M,F,P,S,T,W,Y,V; X467A,R,N,D,C,Q,E,G,H,I,L,K,M,-
F,P,S,T,W,Y,V; X469A,R,N,D,C,Q,G,H,I,L,K,M,F,P,S,T,W,Y,V;
X473N,Q,G,H,I,L,M,F,P,S,T,W,Y,V; X474A,R,G,H,I,L,M,F,P,W,Y,V;
X475A,N,Q,G,H,I,L,M,F,P,S,T,W,Y,V; X476G,H,I,L,M,F,P,S,T,W,Y,V;
X482A,N,D,C,G,H,I,L,M,F,S,T,W,Y,V; X483A,N,D,C,Q,E,G,H,I,L,M,F,P,S,T,W,Y,- V
wherein (a) the variant has alpha-amylase activity and (b) each position

corresponds to a position of the amino acid sequence of the parent Termamyl-like alpha-amylase having the amino acid sequence shown in SEQ ID NO: 8 (Bacillus licheniformis alph-amylase).

Claims Text - CLTX (3):

3. A variant of a parent Termamyl-like alpha-amylase, comprising one or more of the following substitutions: X7A,R,N,D,C,Q,E,G,H,K,M,P,S,Y,V; X8C,M X9A,R,N,D,C,Q,G,H,M,P,S,T,W,Y,V; X11A,N,D,C,Q,G,H,I,L,M,P,S,T,W,Y,V- ; X12A,R,N,D,C,E,G,H,I,L,K,M,F,P,S,T,W,Y,V; X19A,R,N,D,C,Q,E,G,H,I,L,K,M,F-,P,S,T,W,Y,V; X21A,R,N,D,C,Q,E,G,H,I,L,K,M,F,P,S,T,W,Y,V; X22A,R,N,D,C,Q,E,G,H,I,L,K,M,F,P,S,T,W,Y,V; X25A,R,N,D,C,Q,E,G,H,I,L,K,M,-F,P,S,T,W,Y,V; X32A,R,N,D,C,Q,E,G,H,I,L,K,M,F,P,S,T,W,Y,V; X40A,R,N,D,C,Q,E,G,H,I,L,K,M,F,P,S,T,W,Y,V; X41A,R,N,D,C,Q,E,G,H,I,L,K,M,-F,P,S,T,W,Y,V; X46A,R,D,C,G,K,M,P,W,Y; X48R,N,D,C,Q,E,G,H,K,M,F,P,W,Y; X55A,R,N,D,C,Q,E,G,H,I,L,K,M,F,P,S,T,W,Y,V; X57R,N,D,C,Q,E,G,H,K,M,P,W; X58A,R,N,D,C,Q,E,G,H,K,M,S,T,W,Y; X60A,R,D,C,Q,E,G,H,I,L,K,M,F,P,S,T,W,Y,- V; X77A,R,D,C,G,K,M,P,W,Y; X95A,R,N,D,C,Q,E,G,H,I,L,K,M,F,P,S,T,W,Y,V; X97A,R,N,D,C,Q,E,G,H,I,L,K,M,F,P,S,T,W,Y,V; X98A,R,D,C,G,K,M,P,W,Y; X99R,N,D,C,Q,E,G,H,I,L,K,M,F,P,S,T,W,Y,V; X100A,R,D,C,Q,E,G,H,I,K,M,F,P,S-,T,W,Y,V; X101A,N,C,Q,G,I,L,M,P,S,T,W,Y,V; X102N,D,C,Q,E,H,I,L,M,F,P,W,Y,V- ; X103A,N,D,C,Q,E,G,M,P,S,W,Y; X105A,N,C,Q,G,H,I,L,M,P,S,T,Y,V; X107R,N,D,Q,E,H,K,M,F,P,W,Y; X115R,N,D,C,Q,E,H,I,L,K,M,F,P,S,T,W,Y,V; X118R,N,C,Q,E,H,I,L,K,M,F,P,S,T,W,Y,V; X135A,R,N,D,C,Q,E,G,H,I,L,K,M,F,P,-S,T,W,Y,V; X139A,R,N,D,C,Q,E,G,H,I,L,K,M,F,P,S,T,W,Y,V; X141A,R,N,D,C,Q,E,G,H,I,L,K,M,F,P,S,T,W,Y,V; X143A,R,N,D,C,Q,E,G,H,I,L,K,-M,F,P,S,T,Y,V; X151A,R,N,D,C,Q,E,G,H,K,M,P,S,T,Y,V; X159A,R,N,D,C,Q,E,G,H,I,L,K,M,F,P,S,T,W,V; X160A,R,N,D,C,Q,E,G,H,I,L,K,M,-F,P,S,T,W,Y,V; X161A,R,D,C,Q,E,G,H,I,L,K,M,F,P,S,T,W,Y,V; X162A,R,N,D,C,Q,E,G,H,I,L,K,M,F,P,S,T,W,Y,V; X163A,R,N,D,C,Q,E,G,H,I,L,K,-M,F,P,S,T,W,Y,V; X166A,R,N,D,C,Q,G,H,I,L,K,M,F,P,S,T,W,Y,V; X175A,R,D,C,G,K,M,P,W,Y; X177A,N,D,C,Q,E,H,I,L,K,M,P,S,T,W,Y,V; X183A,R,D,C,Q,E,G,H,I,L,K,M,F,P,S,T,W,Y,V; X186A,R,N,D,C,Q,E,G,H,I,L,K,M,-F,P,S,T,W,Y,V; X187A,R,C,Q,E,G,H,I,L,K,M,F,P,W,Y,V; X192A,R,N,D,C,Q,E,G,H,I,L,K,M,F,P,S,T,W,Y,V; X199R,N,D,C,Q,E,G,H,I,L,K,M,-F,P,S,T,W,Y,V; X200A,R,D,C,Q,E,G,H,I,L,K,M,F,P,S,T,W,Y,V; X202A,R,D,C,Q,E,.G,H,I,L,K,M,F,P,S,T,W,Y,V; X203A,R,D,C,G,K,M,P; X208A,R,N,D,C,Q,E,G,H,L,K,M,F,P,S,T,W,Y,V; X212A,N,C,Q,G,H,M,P,S,T,V; X215A,R,N,D,C,Q,E,G,H,I,L,K,M,F,P,S,T,W,Y,V; X219A,R,D,C,G,K,M,P,W,Y; X228A,R,N,D,C,Q,E,G,H,I,L,K,M,P,S,T,W,Y,V; X230A,R,N,D,C,Q,E,G,L,M,P,S,T,-W,Y,V; X233R,N,C,Q,E,G,H,I,K,M,P,S,T,W,Y; X236A,C,Q,G,H,I,M,P,S,T,V; X238A,R,N,D,C,Q,E,G,H,I,K,M,P,S,T,W,Y,V; X240A,R,N,D,C,Q,E,G,H,I,L,K,M,F,-P,S,T,W,Y,V; X241A,N,C,Q,G,H,P,S,T,V; X244A,R,N,D,C,Q,E,G,H,I,L,K,M,F,P,S,-T,W,Y,V; X248A,R,N,D,C,Q,E,G,H,I,L,K,M,F,P,S,T,W,Y,V; X256C,M; X258A,R,N,D,C,Q,E,G,H,I,L,K,M,F,P,S,T,W,Y,V; X259A,R,N,D,C,Q,E,G,H,K,M,P,-S,T,W,Y,V; X260R,N,D,C,Q,E,H,I,L,K,M,F,P,T,W,Y,V; X262A,R,D,C,G,K,M,P,W,Y; X270A,N,C,Q,G,I,L,M,F,P,S,T,W,Y,V; X273A,R,D,C,G,K,M,P,Y; X274A,R,N,D,C,Q,E,G,H,I,L,K,M,F,P,S,T,W,Y,V; X277A,R,N,D,C,Q,E,G,H,K,M,P,-S,W,Y,V; X281A,R,N,D,C,Q,E,G,K,M,P,S,T,W,Y,V; X283A,R,N,C,Q,E,G,I,L,K,M,F,-P,S,T,W,Y,V; X284A,R,N,D,C,Q,E,G,I,L,K,M,F,P,S,T,Y,V; X285A,R,D,C,Q,E,G,H,I,K,M,F,P,S,T,W,Y,V; X286A,R,D,C,Q,E,G,H,I,K,M,F,P,S,-T,W,Y,V;preferably X286N,C,Q,I,L,M,P,T,V,Y,F; X287R,N,D,C,Q,E,G,H,I,L,K,M,-F,P,S,T,W,Y,V; X288A,R,N,D,C,Q,E,G,H,I,L,K,M,F,P,S,T,W,Y,V; X289A,R,N,D,C,Q,E,G,H,I,L,K,M,F,P,S,T,W,Y,V; X292A,R,N,D,C,Q,E,G,H,I,L,K,-M,F,P,S,T,W,Y,V; X295A,R,N,D,C,Q,E,G,H,I,L,K,M,F,P,S,T,W,Y,V; X296A,R,N,D,C,Q,E,G,H,I,L,K,M,F,P,S,T,W,Y,V; X304C,M; X307A,R,N,D,C,Q,E,G,H,I,L,K,M,F,P,S,T,W,Y,V; X312A,N,C,Q,G,H,I,L,M,F,P,S,-T,W,Y,V; X313A,R,N,D,C,Q,E,G,H,I,L,K,M,F,P,S,T,W,Y,V; X320R,N,D,C,Q,E,G,H,I,L,K,M,F,P,S,T,W,Y,V; X322R,N,D,C,Q,E,G,H,L,K,M,F,P,-S,T,W,Y,V; X323A,R,N,D,C,Q,E,G,I,L,K,M,F,P,S,T,W,Y,V;

X325A,R,D,C,Q,E,G,H,I,L,K,M,F,P,S,T,W,Y,V; X326A,R,N,C,Q,E,G,M,P,S,T,W; X327A,R,C,G,H,I,L,K,M,P,S,T,W,Y,V; X329A,R,N,D,C,Q,E,G,H,I,L,K,M,F,P,T,W,-Y,V; X331N,D,C,Q,E,G,H,I,L,M,F,P,S,T,W,Y,V; X339A,R,N,C,Q,E,G,H,I,L,K,M,F,-P,S,T,W,Y,V; X343A,R,N,D,C,Q,E,G,H,I,L,K,M,F,P,S,T,Y,V; X344A,R,N,D,C,Q,E,G,H,I,L,K,M,F,P,S,T,W,Y,V; X346A,R,N,D,Q,E,G,H,I,L,K,M,-F,P,S,T,W,Y,V; X347A,R,N,D,C,Q,E,G,H,I,L,K,M,F,P,S,T,W,Y,V; X349R,N,D,Q,E,G,H,I,L,K,M,F,P,S,T,W,Y,V; X350A,R,N,C,Q,G,H,I,L,K,M,F,P,S,-T,Y,V; X359R,N,D,C,Q,E,G,H,I,L,K,M,F,P,S,T,W,Y,V; X360N,D,Q,G,H,I,L,M,F,P,-S,T,W,Y,V; X369A,R,N,D,C,Q,E,G,H,I,L,K,M,F,P,S,T,W,Y,V; X377A,R,N,D,C,Q,E,G,H,I,L,K,M,F,P,S,T,W,Y,V; X380A,R,N,D,C,Q,E,G,H,K,P,S,-W,Y,V; X387A,R,N,D,C,Q,E,G,H,L,K,M,P,S,T,W,Y,V; X409N,C,Q,E,G,H,M,P,S,T,W,-Y,V; X410A,R,N,D,C,Q,E,G,H,I,L,K,M,F,P,S,T,W,Y,V; X411A,R,N,D,C,Q,E,G,H,I,-L,K,M,F,P,S,T,W,Y,V; X412R,N,C,Q,E,G,H,I,L,K,M,F,P,S,T,W,Y,V; X423A,R,N,D,C,Q,E,G,H,I,L,K,M,F,P,S,T,W,Y,V; X424A,R,N,D,C,Q,E,G,H,I,L,K,-M,F,P,S,T,W,Y,V; X426A,R,N,D,C,Q,E,G,H,I,L,K,M,F,P,S,T,W,Y,V; X427A,R,N,D,C,Q,E,G,H,K,M,P,S,T,Y,V; X428A,N,D,Q,E,G,H,I,M,F,P,S,W,Y,V; X429A,R,N,D,C,Q,E,G,H,I,L,K,M,F,P,S,T,W,Y,V; X430A,R,D,C,Q,E,G,H,I,L,K,M,-F,P,S,T,W,Y,V; X438C,M; X440R,N,D,C,Q,E,G,H,I,L,K,M,F,P,S,T,W,Y,V; X441A,R,N,D,C,Q,E,G,H,I,L,K,M,F,P,S,T,W,Y,V; X449A,R,N,D,C,Q,E,G,H,I,L,K,-M,F,P,S,T,W,Y,V; X462A,R,N,D,C,Q,E,G,H,I,L,K,M,F,P,S,T,W,Y,V; X472A,R,N,D,C,Q,E,G,H,I,L,K,M,F,P,S,T,W,Y,V; X477A,R,N,D,C,Q,E,G,H,I,L,K,-M,F,P,S,T,W,Y,V; X479A,R,N,D,Q,E,G,H,I,L,K,M,F,P,S,W,Y,V; X480A,R,D,C,G,K,M,P,W,Y; X481A,R,N,D,C,Q,E,G,H,K,M,P,S,T,Y,V wherein (a) the variant has alpha-amylase activity and (b) each position corresponds to a position of the amino acid sequence of the parent Termamyl-like alpha-amylase having the amino acid sequence shown in SEQ ID NO: 8 (Bacillus licheniformis alpha-amylase).

Claims Text - CLTX (4):

4. A variant of a parent Termamyl-like alpha-amylase, comprising an alteration at one or more positions selected from the group of: A1 insertion; L3 insertion; N4 insertion; N17 insertion; D18 insertion; Q20 insertion; R23 insertion; R24 insertion; D28 insertion; Y56 insertion; L61 insertion or deletion; Y62 insertion; F67 insertion or deletion; H68 insertion; K80 insertion or deletion; G81 insertion or deletion; Q84 insertion; S85 insertion; H91 insertion or deletion; S92 insertion or deletion; K106 insertion or deletion; D110 insertion or deletion; D114 deletion; E119 insertion or deletion; D121 insertion; P122 insertion; A123 insertion; D124 insertion; R125 insertion; N126 insertion; R127 insertion; I129 insertion; G131 insertion; L134 insertion; K136 insertion; N172 insertion; E185 insertion; L196 insertion or deletion; P206 insertion or deletion; T217 insertion; W218 insertion; D231 insertion or deletion; A232 insertion or deletion; H235 insertion or deletion; N246 insertion; H247 insertion; R249 insertion; K251 insertion; F257 insertion or deletion; N278 insertion; G310 insertion or deletion; H316 insertion; P317 insertion; D328 insertion or deletion; G332 insertion or deletion; E355 insertion or deletion; Y358 insertion; Y363 insertion; Y367 insertion; K370 insertion; S373 insertion; R375 insertion; E376 insertion; K381 insertion; H382 insertion; R391 insertion or deletion; Y396 insertion; R413 insertion or deletion; E414 insertion or deletion; G415 insertion or deletion; D416 insertion; S417 insertion; S418 insertion; V419 insertion; A420 insertion; N421 insertion; S422 insertion or deletion; Y439 insertion; A445 insertion or deletion; G446 insertion or deletion; T448 insertion or deletion; H450 insertion; G454 insertion or deletion; N455 insertion; E458 insertion; P459 insertion; V460 insertion; V461 insertion; N463 insertion; S464 insertion; E465 insertion; W467 insertion; wherein (a) the alteration(s) are independently (as specified above): (i) an insertion of an amino acid downstream of the amino acid which occupies the position, or (ii) a deletion of the amino acid which occupies the position, (b) the variant has alpha-amylase activity and (c) each position

corresponds to a position of the amino acid sequence of the parent Termamyl-like alpha-amylase having the amino acid sequence shown in SEQ ID NO: 8 (Bacillus licheniformis alph-amylase).

Claims Text - CLTX (5):
5. A variant of a parent Termamyl-like alpha-amylase, comprising an alteration at one or more positions selected from the group of: L7 insertion or deletion; M8 insertion; Y10 insertion; F11 insertion; E12 insertion or deletion; M15 insertion; G19 insertion; H21 insertion; W22 insertion; L25 insertion; V40 insertion or deletion; W41 insertion; P43 insertion or deletion; P44 insertion or deletion; Y46 insertion; G55 insertion; Y59 insertion; Y77 insertion; G78 insertion or deletion; L90 insertion or deletion; I95 insertion; V97 insertion; Y98 insertion; G99 insertion; D100 insertion; V101 insertion; V102 insertion; H105 insertion or deletion; A109 insertion or deletion; V115 insertion or deletion; V118 insertion or deletion; I135 insertion; T139 insertion or deletion; F141 insertion or deletion; Y195 insertion; V208 insertion or deletion; W215 insertion; Y219 insertion; I236 insertion or deletion; F238 insertion or deletion; F240 insertion or deletion; W244 insertion; V248 insertion; M256 insertion; T258 insertion or deletion; V259 insertion or deletion; V312 insertion or deletion; V313 insertion or deletion; S320 insertion; T322 insertion or deletion; F323 insertion or deletion; D325 insertion or deletion; N326 insertion; H327 insertion or deletion; Q330 insertion or deletion; P331 insertion or deletion; Y348 insertion; A349 insertion or deletion; F350 insertion or deletion; P359 insertion or deletion; Q360 insertion; D365 insertion or deletion; M366 insertion; T369 insertion; I377 insertion; I384 insertion or deletion; L388 insertion or deletion; G423 insertion or deletion; L424 insertion or deletion; M438 insertion; G441 insertion or deletion; W449 insertion; I462 insertion; I479 insertion or deletion; Y480 insertion; V481 insertion or deletion; wherein (a) the alteration(s) are independently (as specified above): (i) an insertion of an amino acid downstream of the amino acid which occupies the position, or (ii) a deletion of the amino acid which occupies the position, (b) the variant has alpha-amylase activity and (c) each position corresponds to a position of the amino acid sequence of the parent Termamyl-like alpha-amylase having the amino acid sequence shown in SEQ ID NO: 8 (Bacillus licheniformis alph-amylase).

Claims Text - CLTX (6):
6. The variant of any of claims 1-5, wherein the parent Termamyl-like alpha-amylase is derived from a strain of B. licheniformis (SEQ ID NO: 8), B. amyloliquefaciens (SEQ ID NO: 10), B. stearothermophilus (SEQ ID NO: 6), Bacillus sp. (SEQ ID NO: 12 (M560), Bacillus sp. (SEQ ID NO: 2 (SP690)); Bacillus sp. (SEQ ID NO: 4 (SP722); Bacillus sp. #707 alpha-amylase (SEQ ID NO: 13); KSM-AP1378.

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TITLE: Alpha-amylase variant with altered properties

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INVENTOR-INFORMATION:

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PA	2000 01533	2000PA-2000 01533	October 13, 2000
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ABSTRACT:

The present invention relates to variants of parent alpha-amylases, which variant has alpha-amylase activity and exhibits an alteration in at least one of the following properties relative to said parent alpha-amylase: substrate specificity, substrate binding, substrate cleavage pattern, thermal stability, pH/activity profile, pH/stability profile, stability towards oxidation, specific activity, and altered pI, in particular higher pI.

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Summary of Invention Paragraph - BSTX (2):
[0001] The present invention relates to variants (mutants) of parent alpha-amylases, in particular of Bacillus origin, which variant has alpha-amylase activity and exhibits an alteration in at least one of the following properties relative to said parent alpha-amylase: substrate specificity, substrate binding, substrate cleavage pattern, thermal stability, pH/activity profile, pH/stability profile, stability towards oxidation, specific activity, and pI, in particular higher pI.

Detail Description Paragraph - DETX (2):
[0032] The object of the present invention is to provide an alpha-amylases,

in particular of Bacillus origin, which variants has alpha-amylase activity and exhibits an alteration in at least one of the following properties relative to said parent alpha-amylase: substrate specificity, substrate binding, substrate cleavage pattern, thermal stability, pH/activity profile, pH/stability profile, stability towards oxidation, specific activity, and altered pI, in particular higher pI.

Detail Description Paragraph - DETX (53):
[0077] Variants of the invention may have altered oxidation stability, in particular higher oxidation stability, in comparison to the parent alpha-amylase.

Detail Description Paragraph - DETX (77):
[0097] Important positions and mutations with respect to obtaining variants with improved stability at low pH are Aspargine substitutions. Preferred mutations include substitution or deletion of one or more Aspargine (Asn). Target Aspargines in SEQ ID NO: 2 (KSM-36) are N4, N17, N23, N34, N49, N68, N93, N96, N104, N121, N124, N147, N148, N161, N172, N179, N181, N183, N190, N192, N200, N278, N289, N291, N306, N326, N360, N371, N373, N393, N421, N430, N455, N463, N473, N482, which may be substituted with any other amino acid, or deleted, in particular N190F.

Detail Description Paragraph - DETX (93):
[0109] In relation to the above, a further aspect of the present invenbon relates to a method for generating a variant of a parent alpha-amylase, e.g. wherein the variant exhibits altered or increased thermal stability relative to the parent, the method comprising:

Detail Description Paragraph - DETX (96):
[0112] (c) screening for host cells expressing an alpha-amylase variant which has an altered property (e.g., pH-stability) relative to the parent alpha-amylase.

Detail Description Paragraph - DETX (117):
[0129] In the vector, the DNA sequence should be operably connected to a suitable promoter sequence. The promoter may be any DNA sequence, which shows transcriptional activity in the host cell of choice and may be derived from genes encoding proteins either homologous or heterologous to the host cell. Examples of suitable promoters for directing th transcription of the DNA sequence encoding an alpha-amylase variant of the invention, especially in a bacterial host, are the promoter of the lac operon of E.coli, the Streptomyces coelicolor agarase gene dagA promoters, the promoters of the Bacillus licheniformis alpha-amylase gene (amyL), the promoters of the Bacillus stearothermophilus maltogenic amylase gene (amyM), the promoters of the Bacillus amyloliquefaciens alpha-amylase (amyQ), the promoters of the Bacillus subtilis xylA and xylB genes etc. For transcription in a fungal host, examples of useful promoters are those derived from the gene encoding A. oryzae TAKA amylase, Rhizomucor miehei aspartic proteinase, A. niger neutral alpha-amylase, A. niger acid stable alpha-amylase, A. niger glucoamylase, Rhizomucor miehei lipase, A. oryzae alkaline protease, A. oryzae triose phosphate isomerase or A. nidulans acetamidase.

Detail Description Paragraph - DETX (198):
[0186] Amylases: Suitable amylases (alpha and/or beta) include those of bacterial or fungal origin. Chemically modified or protein engineered mutants are included. Amylases include, for example, alpha-amylases obtained from Bacillus, e.g., a special strain of B. licheniformis, described in more detail in GB 1,296,839. Examples of useful alpha-amylases are the variants described in WO 94/02597, WO 94/18314, WO 96/23873, and WO 97/43424, especially the

variants with substitutions in one or more of the following positions: 15, 23, 105, 106, 124, 128, 133, 154, 156, 181, 188, 190, 197, 202, 208, 209, 243, 264, 304, 305, 391, 408, and 444.

Detail Description Paragraph - DETX (253):

[0233] The assay can be used to screening of alpha-amylase variants having an improved stability at high pH compared to the parent enzyme and alpha-amylase variants having an improved stability at high pH and medium temperatures compared to the parent enzyme depending of the screening temperature setting.

Detail Description Paragraph - DETX (304):

[0270] 3. Decide on which kind of mutations should be carried out, e.g. with respect to the desired stability and/or performance of the variant to be constructed

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ABSTRACT:

The invention relates to a method of producing ethanol by fermentation, said method comprising a secondary liquefaction step in the presence of a thermostable acid alpha-amylase or, a thermostable maltogenic acid alpha-amylase.

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Detail Description Paragraph - DETX (100):
[0108] The "primary liquefaction" is preferably performed in the presence of an alpha-amylase, e.g., derived from a micro-organism or a plant. Preferred alpha-amylases are of fungal or bacterial origin. Bacillus alpha-amylases (often referred to as "Termamyl-like alpha-amylases"), variant and hybrids thereof, are specifically contemplated according to the invention. Well-known Termamyl-like alpha-amylases include alpha-amylase derived from a strain of B. licheniformis (commercially available as Termamyl.TM.), B. amyloliquefaciens, and B. stearothermophilus alpha-amylase. Other Termamyl-like alpha-amylases include alpha-amylase derived from a strain of the Bacillus sp. NCIB 12289, NCIB 12512, NCIB 12513 or DSM 9375, all of which are described in detail in WO 95/26397, and the alpha-amylase described by Tsukamoto et al., Biochemical and

Biophysical Research Communications, 151 (1988), pp. 25-31. In the context of the present invention a Termamyl-like alpha-amylase is an alpha-amylase as defined in WO 99/19467 on page 3, line 18 to page 6, line 27. Contemplated variants and hybrids are described in WO 96/23874, WO 97/41213, and WO 99/19467, and include the Bacillus stearothermophilus alpha-amylase (BSG alpha-amylase) variant, alpha-amylase TTC, having the following mutations delta(181-182)+N193F (also denoted I181*+G182*+N193F) compared to the wildtype amino acid sequence set forth in SEQ ID NO:3 disclosed in WO 99/19467. Contemplated alpha-amylase derived from a strain of Aspergillus includes Aspergillus oryzae and Aspergillus niger alpha-amylases.

Detail Description Paragraph - DETX (133):

[0141] Other contemplated Aspergillus glucoamylase variants include variants to enhance the thermal stability: G137A and G139A (Chen et al. (1996), Prot. Engng. 9, 499-505); D257E and D293E/Q (Chen et al. (1995), Prot. Engng. 8, 575-582); N182 (Chen et al. (1994), Biochem. J. 301, 275-281); disulphide bonds, A246C (Fierobe et al. (1996), Biochemistry, 35, 8698-8704; and introduction of Pro residues in position A435 and S436 (Li et al. (1997), Protein Engng. 10, 1199-1204. Furthermore, Clark Ford presented a paper on Oct. 17, 1997, ENZYME ENGINEERING 14, Beijing/China Oct 12-17, 1997, Abstract number: Abstract book p.0-61. The abstract suggests mutations in positions G137A, N20C/A27C, and S30P in an Aspergillus awamori glucoamylase to improve the thermal stability. Other glucoamylases include Talaromyces glucoamylases, in particular derived from Talaromyces emersonii (WO 99/28448), Talaromyces leycettanus (U.S. Pat. No. Re. 32,153), Talaromyces duponti, Talaromyces thermopiles (U.S. Pat. No. 4,587,215). Bacterial glucoamylases contemplated include glucoamylases from the genus Clostridium, in particular C. thermoamylolyticum (EP 135,138), and C. thermohydrosulfuricum (WO 86/01831).

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DOCUMENT-IDENTIFIER: US 20040082028 A1

TITLE: Pullulanase variants and methods for preparing such variants with predetermined properties

PUBLICATION-DATE: April 29, 2004

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
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APPL-NO: 09/ 996024

DATE FILED: November 16, 2001

RELATED-US-APPL-DATA:

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parent division-of 09514599 20000228 US GRANTED

parent-patent 6350599 US

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DK	PA 2000 00045	2000DK-PA 2000 00045	January 28, 2000

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ABSTRACT:

The present invention relates to pullulanase variants, wherein the variants have improved properties, for example, altered pH optimum, improved thermostability, altered substrate specificity, increased specific activity or altered cleavage pattern. The present invention also relates to methods of making pullulanase variants having at least one altered property based on the three-dimensional structure of a parent pullulanase.

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application is a division of U.S. application Ser. No. 09/514,599 filed Feb. 28, 2000 and claims, under 35 U.S.C. 119, priority of Danish application no. PA 2000 00045 filed Jan. 12, 2000, the contents of which are fully incorporated herein by reference.

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Abstract Paragraph - ABTX (1):

The present invention relates to pullulanase variants, wherein the variants have improved properties, for example, altered pH optimum, improved thermostability, altered substrate specificity, increased specific activity or altered cleavage pattern. The present invention also relates to methods of making pullulanase variants having at least one altered property based on the

three-dimensional structure of a parent pullulanase.

Detail Description Paragraph - DETX (54):
[0084] In one embodiment, the pullulanase variant of the invention has an improved thermostability (and/or the method of the invention provides a pullulanase with an improved thermostabilty) as defined by differential scanning calorimetry (DSC) using the method described herein.

Detail Description Paragraph - DETX (55):
[0085] In another embodiment, the pullulanase variant of the invention has an improved thermostability (and/or the method of the invention provides a pullulanase with an improved thermostabilty) as defined by an increased half-time (T.sub.1/2) of at least about 5%, preferably at least about 10%, more preferably at least about 15%, more preferably at least about 25%, most preferably at least about 50%, such as at least about 100%, in the "T.sub.1/2 assay for liquefaction" described herein, using a pH of 5.0 and a temperature of 95.degree. C. Pullulanase variants according to this definition are suitable for use in the liquefaction step of the starch conversion process.

Detail Description Paragraph - DETX (57):
[0087] In a further embodiment, the enzyme variant of the invention has an improved thermostability (and/or the method of the invention provides a pullulanase with an improved thermostabilty) as defined by an increased half-time (T.sub.1/2) of at least about 5%, preferably at least about 10%, more preferably at least about 15%, more preferably at least about 25%, most preferably at least about 50%, such as at least about 100%, in the "T.sub.1/2 assay for saccharification" described herein, using a pH of 4.5 and a temperature of 70.degree. C. Such variants are suitable for use in the saccharification step of the starch conversion process.

Detail Description Paragraph - DETX (93):
[0123] Pullulanase Variants with Altered Stability

Detail Description Paragraph - DETX (94):
[0124] A variant with improved stability (typically increased thermostability) may be obtained by substitution with proline, substitution of histidine with another amino acid, introduction of a disulfide bond, removal of a deamidation site, altering a hydrogen bond contact, filling in an internal structural cavity with one or more amino acids with bulkier side groups, introduction of interdomain interactions, altering charge distribution, helix capping, or introduction of a salt bridge.

Detail Description Paragraph - DETX (123):
[0153] Furthermore, it is envisaged from the structure that deletion of certain amino acid residues will confer increased stability, such as increased thermostability, to the thus produced variant. Variants, which are believed to be of particular importance, comprises a deletion of amino acid residues corresponding to the following residues of the amino acid sequence set forth in SEQ ID NO: 1:

Detail Description Paragraph - DETX (125):
[0155] Other deletions which are expected to confer increased stability, such as increased thermostability, to the pullulanase variant comprises a deletion of amino acid residues corresponding to the following residues of the amino acid sequence set forth in SEQ ID NO: 1:

Detail Description Paragraph - DETX (127):
[0157] Furthermore, the following deletions are expected to confer increased stability, such as increased thermostability, to the pullulanase variant

comprises a deletion of amino acid residues corresponding to the following residues of the amino acid sequence set forth in SEQ ID NO: 1:

Detail Description Paragraph - DETX (130):
[0160] For example, it is envisaged that deletion of the below amino acid residues will confer increased stability, such as increased thermostability, to the thus produced variant of the pullulanase from Bacillus deramificans (SEQ ID NO: 3):

Detail Description Paragraph - DETX (132):
[0162] Other deletions which are expected to confer increased stability, such as increased thermostability, to the pullulanase variant comprises a deletion of amino acid residues corresponding to the following residues of the amino acid sequence set forth in SEQ ID NO: 3:

Detail Description Paragraph - DETX (134):
[0164] Furthermore, the following deletions are expected to confer increased stability, such as increased thermostability, to the pullulanase variant comprises a deletion of amino acid residues corresponding to the following residues of the amino acid sequence set forth in SEQ ID NO: 3:

Detail Description Paragraph - DETX (147):
[0177] f) testing the stability and/or the temperature dependent activity profile of the variant; and

Detail Description Paragraph - DETX (149):
[0179] h) selecting a variant having increased stability and/or an altered temperature dependent activity profile as compared to the parent pullulanase.

Detail Description Paragraph - DETX (150):
[0180] In a preferred embodiment of the invention the variant pullanase provided by the above method have increased thermostability as compared to the parent pullulanase. The thermostability of a given variant may be assessed as described in the above section entitled "Methods for determining stability, activity and specificity".

Detail Description Paragraph - DETX (162):
[0192] A variant with improved stability (typically improved thermostability) as compared to the parent pullulanase may be obtained by introducing new interdomain and intradomain contacts, such as establishing inter- or intradomain disulfide bridges.

Detail Description Paragraph - DETX (169):
[0199] f) testing the stability of said variant; and

Detail Description Paragraph - DETX (171):
[0201] h) selecting a variant having increased stability as compared to the parent pullulanase.

Detail Description Paragraph - DETX (172):
[0202] In a preferred embodiment of the invention the variant pullanase provided by the above method have increased thermostability as compared to the parent pullulanase. The thermostability of a given variant may be assessed as described in the above section entitled "Methods for determining stability, activity and specificity".

Detail Description Paragraph - DETX (179):
[0209] A variant with improved stability (typically improved thermostability) as compared to the parent pullulanase may be obtained by

changing the surface charge distribution of the pullulanase. For example, when the pH is lowered to about 5 or below histidine residues typically become positively charged and, consequently, unfavorable electrostatic interactions on the protein surface may occur. By engineering the surface charge of the pullulanase one may avoid such unfavorable electrostatic interactions which in turn leads to a higher stability of the pullulanase.

Detail Description Paragraph - DETX (186):
[0216] f) testing the stability of said variant; and

Detail Description Paragraph - DETX (188):
[0218] h) selecting a variant having increased stability as compared to the parent pullulanase.

Detail Description Paragraph - DETX (196):
[0226] In a preferred embodiment of the invention the variant pullulanase provided by the above method(s) have increased thermostability as compared to the parent pullulanase. The thermostability of a given variant may be assessed as described in the above section entitled "Methods for determining stability, activity and specificity".

Detail Description Paragraph - DETX (208):
[0238] Variants with improved stability, in particular variants with improved thermostability, can be obtained by improving existing or introducing new interdomain or intradomain contacts. Such improved stability can be achieved by the modifications listed below.

Detail Description Paragraph - DETX (209):
[0239] Thus, one preferred embodiment of the invention relates to a variant of a parent pullulanase which has an improved stability and one or more salt bridges as compared to the parent pullulanase, wherein said variant comprises a modifications, e.g. a substitution, in a position corresponding to at least one of the following sets of positions in SEQ ID NO: 1:

Detail Description Paragraph - DETX (385):
[0415] In relation to the above, a further aspect of the present invention relates to a method for generating a variant of a parent pullulanase, wherein the variant exhibits an altered property, such as increased thermostability, increased stability at low pH and at low calcium concentration, relative to the parent pullulanase, the method comprising:

Detail Description Paragraph - DETX (407):
[0437] 3. Decide on which kind of mutations should be carried out, e.g. with respect to the desired stability and/or performance of the variant to be constructed

Detail Description Paragraph - DETX (420):
[0450] In the vector, the DNA sequence should be operably connected to a suitable promoter sequence. The promoter may be any DNA sequence which shows transcriptional activity in the host cell of choice and may be derived from genes encoding proteins either homologous or heterologous to the host cell. Examples of suitable promoters for directing the transcription of the DNA sequence encoding a pullulanase variant of the invention, especially in a bacterial host, are the promoter of the lac operon of E.coli, the Streptomyces coelicolor agarase gene dagA promoters, the promoters of the Bacillus licheniformis .alpha.-amylase gene (amyL), the promoters of the Bacillus stearothermophilus maltogenic amylase gene (amyM), the promoters of the Bacillus amyloliquefaciens .alpha.-amylase (amyQ), the promoters of the Bacillus subtilis xylA and xylB genes, etc. For transcription in a fungal host,

examples of useful promoters are those derived from the gene encoding A. oryzae TAKA amylase, Rhizomucor miehei aspartic proteinase, A. niger neutral .alpha.-amylase, A. niger acid stable .alpha.-amylase, A. niger glucoamylase, Rhizomucor miehei lipase, A. oryzae alkaline protease, A. oryzae triose phosphate isomerase or A. nidulans acetamidase.

Detail Description Paragraph - DETX (436):

[0466] To screen for variants with increased stability, the filter with bound pullulanase variants can be pretreated prior to the detection step described above to inactivate variants that do not have improved stability relative to the parent pullulanase. This inactivation step may consist of, but is not limited to, incubation at elevated temperatures in the presence of a buffered solution at any pH from pH 2 to 12, and/or in a buffer containing another compound known or thought to contribute to altered stability e.g., surfactants, EDTA, EGTA, wheat flour components, or any other relevant additives. Filters so treated for a specified time are then rinsed briefly in deionized water and placed on plates for activity detection as described above. The conditions are chosen such that stabilized variants show increased enzymatic activity relative to the parent after incubation on the detection media.

Detail Description Paragraph - DETX (437):

[0467] To screen for variants with altered thermostability, filters with bound variants are incubated in buffer at a given pH (e.g., in the range from pH 2-12) at an elevated temperature (e.g., in the range from 50.degree.-110.degree. C.) for a time period (e.g., from 1-20 minutes) to inactivate nearly all of the parent pullulanase, rinsed in water, then placed directly on a detection plate containing immobilized Cibacron Blue labeled pullulan and incubated until activity is detectable. As will be understood, thermostability and increased isoamylase activity may be tested simultaneously by using a detection plate containing immobilized Cibacron Red labeled amylopectin and incubate until activity is detectable. Moreover, pH dependent stability can be screened for by adjusting the pH of the buffer in the above inactivation step such that the parent pullulanase is inactivated, thereby allowing detection of only those variants with increased stability at the pH in question. To screen for variants with increased calcium-dependent stability, calcium chelators, such as ethylene glycol-bis(.beta.-aminoethyl ether) N,N,N',N'-tetraacetic acid (EGTA), is added to the inactivation buffer at a concentration such that the parent pullulanase is inactivated under conditions further defined, such as buffer pH, temperature or a specified length of incubation.

Detail Description Paragraph - DETX (438):

[0468] The variants of the invention may be suitably tested by assaying the pullulan- or amylopectin-degrading activity of the variant, for instance by growing host cells transformed with a DNA sequence encoding a variant on a starch-containing agarose plate and identifying pullulan- and/or amylopectin-degrading host cells as described above. Further testing in regard to altered properties, including specific activity, substrate specificity, cleavage pattern, thermoactivation, thermostability, pH dependent activity or optimum, pH dependent stability, temperature dependent activity or optimum, transglycosylation activity, stability, and any other parameter of interest, may be performed on purified variants in accordance with methods known in the art as described below.

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PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20040072718 A1

TITLE: Laundry detergent compositions comprising zwitterionic polyamines and mid-chain branched surfactants

PUBLICATION-DATE: April 15, 2004

INVENTOR-INFORMATION:

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APPL-NO: 10/ 679917

DATE FILED: October 6, 2003

RELATED-US-APPL-DATA:

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child 09980799 20011203 US

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non-provisional-of-provisional 60144113 19990716 US

non-provisional-of-provisional 60144111 19990716 US

US-CL-CURRENT: 510/499

ABSTRACT:

The present invention relates to laundry detergent compositions which provide enhance hydrophilic soil cleaning benefits, said compositions comprising:
a) from about 0.01% by weight of a zwitterionic polyamine;
b) from about 0.01% by weight, of a surfactant system comprising:
i) from 0% to 80% by weight, of a mid-chain branched alkyl sulfate surfactant;
ii) from 0% to 80% by weight, of a mid-chain branched aryl sulfonate

surfactant;
iii) optionally from 0.01% by weight, of a surfactant selected from the group consisting of anionic, nonionic, cationic, zwitterionic, ampholytic surfactants, and mixtures thereof;
c) the balance carriers and other adjunct ingredients.

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Detail Description Paragraph - DETX (136):
[0261] A preferred protease enzyme for use in the present invention is a variant of Protease A (BPN') which is a non-naturally occurring carbonyl hydrolase variant having a different proteolytic activity, stability, substrate specificity, pH profile and/or performance characteristic as compared to the precursor carbonyl hydrolase from which the amino acid sequence of the variant is derived. This variant of BPN' is disclosed in EP 130,756 A, Jan. 9, 1985. Specifically Protease A-BSV is BPN' wherein the Gly at position 166 is replaced with Asn, Ser, Lys, Arg, His, Gln, Ala, or Glu; the Gly at position 169 is replaced with Ser; the Met at position 222 is replaced with Gln, Phe, Cys, His, Asn, Glu, Ala or Thr; or alternatively the Gly at position 166 is replaced with Lys, and the Met at position 222 is replaced with Cys; or alternatively the Gly at position 169 is replaced with Ala and the Met at position 222 is replaced with Ala.

Detail Description Paragraph - DETX (138):
[0263] A preferred protease enzyme for use in the present invention is Protease B. Protease B is a non-naturally occurring carbonyl hydrolase variant having a different proteolytic activity, stability, substrate specificity, pH profile and/or performance characteristic as compared to the precursor carbonyl hydrolase from which the amino acid sequence of the variant is derived. Protease B is a variant of BPN' in which tyrosine is replaced with leucine at position +217 and as further disclosed in EP 303,761 A, Apr. 28, 1987 and EP 130,756 A, Jan. 9, 1985.

Detail Description Paragraph - DETX (163):
[0287] Amylases suitable herein include, for example, .alpha.-amylases described in GB 1,296,839 to Novo; RAPIDASE.RTM., International Bio-Synthetics, Inc. and TERMAMYL.RTM., Novo. FUNGAMYL.RTM. from Novo is especially useful. Engineering of enzymes for improved stability, e.g., oxidative stability, is known. See, for example J. Biological Chem., Vol.260, No. 1, June 1985, pp 6518-6521. Certain preferred embodiments of the present compositions can make use of amylases having improved stability in detergents, especially improved oxidative stability as measured against a reference-point of TERMAMYL.RTM. in commercial use in 1993. These preferred amylases herein share the characteristic of being "stability-enhanced" amylases, characterized, at a minimum, by a measurable improvement in one or more of: oxidative stability, e.g., to hydrogen peroxide/tetraacetylethylenediamine in buffered solution at pH 9-10; thermal stability, e.g., at common wash temperatures such as about 60.degree. C.; or alkaline stability, e.g., at a pH from about 8 to about 11, measured versus the above-identified reference-point amylase. Stability can be measured using any of the art-disclosed technical tests. See, for example, references disclosed in WO 9402597. Stability-enhanced amylases can be obtained from Novo or from Genencor International. One class of highly preferred amylases herein have the commonality of being derived using site-directed mutagenesis from one or more of the Baccillus amylases, especially the Bacillus .alpha.-amylases, regardless of whether one, two or multiple amylase strains are the immediate precursors. Oxidative stability-enhanced amylases vs. the aboveidentified reference amylase are preferred for use, especially in bleaching, more preferably oxygen bleaching,

as distinct from chlorine bleaching, detergent compositions herein. Such preferred amylases include (a) an amylase according to the hereinbefore incorporated WO 9402597, Novo, Feb. 3, 1994, as further illustrated by a mutant in which substitution is made, using alanine or threonine, preferably threonine, of the methionine residue located in position 197 of the B.licheniformis alpha-amylase, known as TERMAMYL.RTM., or the homologous position variation of a similar parent amylase, such as B. amyloliquefaciens, B. subtilis, or B. stearothermophilus; (b) stability-enhanced amylases as described by Genencor International in a paper entitled "Oxidatively Resistant alpha-Amylases" presented at the 207th American Chemical Society National Meeting, Mar. 13-17 1994, by C. Mitchinson. Therein it was noted that bleaches in automatic dishwashing detergents inactivate alpha-amylases but that improved oxidative stability amylases have been made by Genencor from B.licheniformis NCIB8061. Methionine (Met) was identified as the most likely residue to be modified. Met was substituted, one at a time, in positions 8, 15, 197, 256, 304, 366 and 438 leading to specific mutants, particularly important being M197L and M197T with the M197T variant being the most stable expressed variant. Stability was measured in CASCADE.RTM. and SUNLIGHT.RTM.; (c) particularly preferred amylases herein include amylase variants having additional modification in the immediate parent as described in WO 9510603 A and are available from the assignee, Novo, as DURAMYL.RTM.. Other particularly preferred oxidative stability enhanced amylase include those described in WO 9418314 to Genencor International and WO 9402597 to Novo. Any other oxidative stability-enhanced amylase can be used, for example as derived by site-directed mutagenesis from known chimeric, hybrid or simple mutant parent forms of available amylases. Other preferred enzyme modifications are accessible. See WO 9509909 A to Novo.

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PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20040063184 A1

TITLE: Fermentation processes and compositions

PUBLICATION-DATE: April 1, 2004

INVENTOR-INFORMATION:

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APPL-NO: 10/ 459143

DATE FILED: June 10, 2003

RELATED-US-APPL-DATA:

non-provisional-of-provisional 60413730 20020926 US

US-CL-CURRENT: 435/161, 435/105

ABSTRACT:

The present invention provides improved fermentation processes, including for use in an ethanol production process. The improved fermentation processes include applying esterases (such as, lipases, phospholipases and cutinases), laccases, phytases and/or proteases to a fermentation process. The improved fermentation process may also involve the addition of various growth stimulators for the fermenting microorganisms, including vitamins and mineral.

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims the benefit of U.S. provisional application No. 60/413,730 filed Sep. 26, 2002, the contents of which are fully incorporated herein by reference.

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Summary of Invention Paragraph - BSTX (62):
[0059] The liquefaction processes are typically carried out using an alpha-amylase. Preferred alphaamylases are of fungal or bacterial origin. More preferably, the alpha-amylase is a Bacillus alpha-amylases, such as, derived from a strain of B. licheniformis, B. amyloliquefaciens, and B. stearothermophilus. Other alpha-amylases include alpha-amylase derived from a strain of the Bacillus sp. NCIB 12289, NCIB 12512, NCIB 12513 or DSM 9375, all of which are described in detail in WO 95/26397, and the alpha-amylase described by Tsukamoto et al., Biochemical and Biophysical Research Communications, 151 (1988), pp. 25-31. Other alpha-amylase variants and hybrids are described in WO 96/23874, WO 97/41213, and WO 99/19467. Other alpha-amylase include alpha-amylases derived from a strain of Aspergillus, such as, Aspergillus oryzae and Aspergillus niger alpha-amylases.

Summary of Invention Paragraph - BSTX (69):
[0066] Other Aspergillus glucoamylase variants include variants to enhance

the thermal stability, such as, G137A and G139A (Chen et al. (1996), Prot. Engng. 9,499-505); D257E and D293E/Q (Chen et al. (1995), Prot. Engng. 8, 575-582); N182 (Chen et al. (1994), Biochem. J. 301, 275281); disulphide bonds, A246C (Fierobe et al. (1996), Biochemistry, 35, 8698-8704; and introduction of Pro residues in position A435 and S436 (Li et al. (1997), Protein Engng. 10, 1199-1204. Other glucoamylases include Talaromyces glucoamylases, in particular, derived from Talaromyces emersonii (WO 99/28448), Talaromyces leycettanus (U.S. Pat. No. Re. 32,153), Talaromyces duponti, Talaromyces thermophilus (U.S. Pat. No. 4,587,215). Bacterial glucoamylases contemplated include glucoamylases from the genus Clostridium, in particular C. thermoamylolyticum (EP 135,138), and C. thermohydrosulfuricum (WO 86/01831).